The Present and the Future of Degradable Dendrimers and Derivatives in Theranostics

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ABSTRACT

Interest in dendrimer-based nanomedicines has been growing recently, as it is possible to precisely manipulate the molecular weight, chemical composition, and surface functionality of dendrimers, tuning their properties according to the desired biomedical application. However, one important concern about dendrimer-based therapeutics remains-the nondegradability under physiological conditions of the most commonly used dendrimers. Therefore, biodegradable dendrimers represent an attractive class of nanomaterials, since they present advantages over conventional nondegradable dendrimers regarding the release of the loaded molecules and the prevention of bioaccumulation of synthetic materials and subsequent cytotoxicity. Here, we present an overview of the state-of-the-art of the design of biodegradable dendritic structures, with particular focus on the hurdles regarding the use of these as vectors of drugs and nucleic acids, as well as macromolecular contrast agents.

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1 INTRODUCTION

Natural and synthetic macromolecular structures have frequently been used as vectors for the delivery of drugs and therapeutic nucleic acids, as well as diagnostic agents. This concept emerged from the hypothesis that these could protect the molecule of interest from undesirable interactions with components of the biological milieu along with improving its solubility. Several carriers have been studied: linear polymers, micellar assemblies, liposomes, polymersomes, and, more recently, dendritic structures (dendrimers and dendrons).(1) The ideal carrier should facilitate high drug loading, long blood circulation time (in the case of the commonly explored intravenous administration), high accumulation in the desired tissue, low toxicity, low immunogenicity, simplicity in its preparation, and, preferably, adequate biodegradability.

Dendritic structures emerged from a new class of highly branched polymers, first synthesized by Voegtle and colleagues in 1978, and coined "cascade molecules".(2) Later on, Denkewalter, Tomalia, Newkome, Frechet, and co-workers further increased the level of complexity of these branched molecules, giving rise to larger dendritic structures that were then renamed "dendrimers".(3) Dendrimers consist of the following: (a) a central core with two or more reactive groups, (b) repeated units or monomers covalently attached to the central core and organized in layers called "generations" (G), and (c) terminal functional groups on their surface. They can be synthesized by two different approaches: divergent(3b) or convergent.(3d)

The most commonly used dendrimers are poly(amido amine) (PAMAM),(3b) poly(propylene imine) (PPI),(2) and poly(lysine)-based dendrimers;(4) currently, all are commercially available. These and other dendrimers have been proposed as promising carriers for drug delivery(5) due to their unique structural characteristics: globular, well-defined, and very branched structure, as well as their monodispersion and controllable nanosize. Moreover, the presence of a high density of terminal functional groups allows the tethering of different ligands and/or drugs in a specific and controllable manner, simulating the multivalency present in different biological systems. This multivalency is the greater virtue of dendrimers: the enhanced effect that stems from presenting lots of several bioactive molecules at the same time and place. Additionally, dendrimers can also cargo a molecule of interest by forming nanosized structures stabilized by noncovalent interactions.

The characteristics of the surface groups of the dendrimers, besides determining predominantly their physicochemical properties, will also determine their biological activity and biocompatibility. For example, cationic dendrimers will more readily interact with the negatively charged surface of the cell membranes, but have also been found to have more cytotoxicity than anionic or neutral ones.(6) A common approach for masking the surface charge and, in general, improving the biocompatibility of dendrimers and/or dendrons, as well as increasing their circulation time in the bloodstream, is to tether to the dendrimers backbone chains of poly(ethylene glycol) (PEG).(7) This strategy was also carried out in the case of DEP docetaxel, a dendrimer with docetaxel attached that is in Phase 1 clinical trials for the treatment of a wide range of solid tumors including breast, lung, and prostate. Other dendrimers have also reached clinical trials: VivaGel, a G4 poly(l-lysine)-based dendrimer, which acts as an antimicrobial agent being applied in the treatment/prevention of a range of sexually transmitted diseases, is in Phase 3 trials; and Gadomer-17, a poly(lysine)-based dendrimer bearing 24 Gd(III)-DOTA chelates (commercial name Dotarem; DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), is in Phase 2 trials for its use as a dendritic contrast agent for diagnosis in magnetic resonance imaging (MRI).

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Despite the progress in the design of dendritic structures with improved features for biomedical application, one of the main drawbacks of the most currently used dendrimers is their nondegradability under physiological conditions that can result in cytotoxicity induced by the accumulation of nondegradable synthetic materials inside cells or in tissues.(8) In vitro studies have shown that dendrimer cytotoxicity is mainly associated with cell membrane disruption and subsequent necrosis/non-apoptotic cell death.(6a, 8d) Numerous works have thoroughly described the effect of dendrimer chemistry, size, and charge on biological membrane integrity.(8c, 9) However, recent reports suggest that, apart from membrane destabilization, toxicity may also arise from impaired oxidative metabolism resulting from mitochondrial dysfunction(10) and changes in endogenous gene expression(11) that ultimately lead to apoptotic cell death.(10, 11) Dendrimer chemistry, charge, and size are features that will also have an impact on in vivo biodistribution and pharmacokinetics.(6a, 8c, 9a) Additionally, in vivo cytotoxicity will also depend on the dose and the administration route.(6a) However, conflicting data can be found in the open literature concerning in vivo testing. Some studies report that PAMAM and PPI dendrimers, especially at low generations, are not as toxic as initially described.(12) However, others have reported toxicity profiles for the same dendrimers.(13) Consequently, a number of teams has been focusing on the design of biodegradable dendritic structures, to overcome these hurdles. The use of biodegradable materials that, under biological environmental conditions, degrade in time into smaller fragments that can be excreted or eliminated through metabolic pathways is expected to overcome the risk of complications associated with the long-term presence of high-molecular-weight compounds.

The development of biodegradable dendritic structures has also been put forward in the context of the design of "smart" controlled delivery systems in which one aims at triggering and/or sustaining the release of a therapeutic agent via the control of the degradation profile of its vector. Inclusively, some authors have proposed the use of degradable dendritic structures as contrasting agents that can overcome long-term Gd(III) tissue accumulation.(14)

Here, we present the state-of-the-art of biodegradable dendritic structures, with particular focus on the ones used in the context of drug and nucleic acid delivery, as well as their use as macromolecular diagnostic agents. The design and synthesis of such structures are presented, as well as the challenges that remain ahead toward their widespread application. Starting from the assumption that at least some animal research is relevant, ethically acceptable and presently not replaceable, some harm to animals in research may be perceived as a 'necessary evil', in particular in face of the moral importance of advancing biomedical knowledge for the benefit of humans and non-humans alike. However, it should nevertheless be reflected upon in which circumstances it may – or may not – be either 'necessary' or 'evil' to kill animals in the context of animal research. In this chapter, we discuss whether killing is inevitable, or morally problematic, as well as to whom this killing matters.

2. GETTING AND TUNING DEGRADABILITY

As in the case of linear polymers, the biodegradability of dendrimers can be achieved by inclusion in their structure of labile bonds to be broken due to a specific biological activity or stimulus. To the best of our knowledge, the majority of efforts have been focused on the development of dendritic architectures (dendrimers, dendrons, and linear–dendritic block copolymers) with hydrolyzable bonds. The functionalities more susceptible to hydrolytic cleavage are based on anhydrides, esters, phosphoesters, carbamates, ethers, and amides. The main factors that control the rate of degradation of dendritic structures are (a) the chemical bond present on or connecting the monomer units; (b) the hydrophobicity of the monomer units—more hydrophilic monomers result in faster degrading

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structures compared to hydrophobic monomers; (c) size—larger dendrimers degrade more slowly compared to smaller ones due to the higher packaging; and (d) the localization of the cleavable linkages, since the hydrolysis of interior linkages leads to faster degradation of the whole dendrimer. With these parameters taken into account, it is expected that one can modulate the properties of biodegradable dendritic structures to achieve the desired degradation rate for a specific application.

The covalent degradation or dendrimer fragmentation could proceed through removal of a certain percentage of surface functionalities, removal of dendritic branches or monomers, or removal of the central core. Therefore, the cleavage of only certain parts of the backbone of the designed dendrimer could lead to its full degradation into low-molecular-weight fragments. Moreover, it is worthwhile mentioning that the degradation of some cleavage polymeric materials (like aliphatic polyesters) could also be accelerated by the presence of superoxide ions (O_2^-) generated in the body fluids and tissues during inflammatory response to foreign materials.(15)

3. BIODEGRADABLE DENDRITIC STRUCTURES

The different types of biodegradable dendritic structures can be classified according to the type of degradable connection or bond, the monomer on which they are based, and the synthetic strategy to prepare them, among others.

Although very few examples of biodegradable dendritic structures not based on ester bonds can be found (see section 3.4), the majority of the dendrimers that are susceptible to degradation under physiological conditions reported so far contain ester bonds as focal points of degradation. Polyester dendrimers represent an attractive class of nanomaterials due to their good biocompatibility and the compromise between their biodegradability trait and the possibility of synthetic manipulation in comparison with other more hydrolytic susceptible structures, such as polyanhydrides. Even so, the synthesis of these nanocarriers is challenging because of the hydrolytic susceptibility of the ester bond.(16) In contrast, polyethers and polyamide-based dendrimers could withstand much wider selection of synthetic manipulations, yet they do not degrade easily in the body but only under severe conditions of hydrolysis. Thus, these dendritic structures may be more prone to long-term accumulation in vivo. Therefore, these types of dendrimers will not be considered in the context of this Review.

Aromatic polyester dendrimers were the first polyester dendrimers to be described in 1992, by Hawker and Fréchet.(17) With this work, they introduced the convergent synthetic route for the synthesis of dendrimers. Despite containing ester linkages, they are aromatic esters, with high hydrolytic stability. Thus, these structures can barely be regarded as biodegradable dendrimers. In 1996 the first study mentioning biodegradable dendrimers was reported, where the enzymatic degradation of aliphatic chiral polyester dendrimers based on (R)-3-hydroxybutanoic acid and trimesic acid was described (Figure 1).(18) Since then, several other polyester dendritic structures have been proposed: 2,2-bis(hydroxymethyl)propanoic acid (bis-HMPA)-derivatives (section 3.1), other aliphatic esters derivatives (section 3.2), and alternating polyesters (section 3.3). In this work, all the aliphatic polyester dendrimers will be considered biodegradable, even in those cases in which authors did not study their degradation profile.

In the following subsections, the design and synthesis of several biodegradable dendritic structures are presented.

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3.1. Monomers Based on bis-HMPA.

Since its first use, 2,2-bis(hydroxymethyl)propanoic acid (bis-HMPA) (Figure 2a) has been the main monomer of choice in the synthesis of biodegradable dentritic structures. Bis-HMPA is commercially available at low cost, and the resulting polyester dendrimers are non-immunogenic, nontoxic, and biodegradable:(19) all very attractive features when envisaging biological applications.

The first report on the synthesis of polyester dendrimers based on bis-HMPA was by Ihre, Hult, and Söderlind (Figure 2c).(20) Since then, a considerable number of research groups proposed several alternative synthetic routes and different precursors using different protecting groups (Figure 2b), with the aim of improving and simplifying the synthesis of this family of biodegradable dendrimers until higher generations with good yields. Additionally, different functionalizations of the core and/or on the surface were reported by several authors to use these dendrimers for different purposes.(21)

In 2002, Gillies and Fréchet described dendrimers consisting of two bis-HMPA dendrons covalently attached as a "bow-tie" ("bow-tie dendrimers", Figure 2d) aiming at water-soluble drug carriers.(22)

In 2004, Vestberg et al. reported the synthesis of bis-HMPA dendrons up to G5 with porphyrins as core.(23) Porphyrins were used because of their potential applications in many areas, such as photodynamic therapy, nanosensors, and so forth.(24) Bis-HMPA dendrimers containing carboranes, which interest resides in their potential use in boron neutron capture therapy in cancer treatment and will be revisited in more detail in section 4, were synthesized by Parrott(21m) and Galie.(25) Gillies and Fréchet reported the synthesis of polyester dendritic bow-ties with a bis(adamantylurea)-glycinylurea system at the focal point of the bow-tie.(26) They prepared self-assembled polyester bow-tie dendrimers with various peripheral groups.

Bis-HMPA polyester dendrimers presenting cyclic carbonates on their periphery were also reported. The attraction of this approach is that carbonate groups react with amines, even in water, yielding bifunctional products easily.(27)

Some studies that combined identical dendrons via the Diels–Alder reaction to furnish symmetrical dendrimers were reported.(28) In 2008, Kose et al. reported the first example of the synthesis of segment block dendrimers using the Diels–Alder-based synthetic strategy in order to get unsymmetrical dendrimers.(29) The cycloaddition is very attractive in dendrimer synthesis, since no reagents are required. Therefore, the resulting dendrimers are free of impurities such as toxic metals, which represent a problem for biological applications.

Several authors reported linear–dendritic block copolymers for different purposes including drug delivery, where the characteristics of biodegradable bis-HMPA dendrons are combined with linear polymers, also biodegradable in some cases. Polyester dendronized polymers (linear polymers having polyester dendrons at each repeating unit) that are expected to present an extended conformation were also reported by Grayson and Fréchet (based on a nondegradable linear polymer: poly(p-hydroxystyrene))(30) and Lee et al. (based on a degradable linear polymer: poly(ε-caprolactone) (PCL)).(31) Due to their high molecular weight and multivalency, these systems are also considered as promising vectors for drug delivery applications. The degradation half-life at 37 °C for the dendronized hydrophilic PCL was 2.5 days at pH 9.0 and 16 days at pH 7.4 (physiological conditions), while no changes in polymer molecular weight was observed under acidic conditions (pH 5.0, endolysosomal conditions).(31) These authors determined that, in fact, the dominant mode of degradation of these

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ester bonds was under mildly basic conditions (pH 9.0). Additionally, the dendronized hydrophilic polymer was found to degrade much faster than the parent hydrophobic polymer.(32)

Connal et al. explored the known "arm first" synthetic strategy to synthesize cross-linked star polymers, with bis-HMPA dendrons at the end of the arms. Bis-HMPA dendrons up to the G5 were synthesized and functionalized at the focal point with a single alkyl halide, capable of initiating polymerization of styrene by atom transfer radical polymerization (ATRP) (Figure 2f).(33).

In 2009, a series of well-defined and amphiphilic dumbbell-shaped triblock copolymers consisting of comb-shaped poly(l-lactide) (PLLA) end arms and linear PEG center block connected using bis-HMPA dendrons, with narrow molecular weight distributions and varied PLLA arm lengths, were presented (Figure 2e).(34) These dendronized linear polymers provide an alternative dendritic architecture with a variety of aspect ratios accessible depending on the length of the PLLA arms and PEG block, and the generation of the dendrons. Efficient regulation of material properties and cell responses was achieved using this series of compounds, that suggests their potential for various regenerative medicine and tissue-engineering applications.(34) Subsequently, a series of well-defined dumbbell-shaped triblock copolymers consisting of linear PEG linked to the focal point of bis-HMPA dendrons and comb-like poly(ε-caprolactone) with different arm lengths were further used to prepare microspheres with potential application as carriers for controlled delivery of water-soluble drugs.(35)

Recently, diblock and triblock dendron–polymer conjugates containing biodegradable bis-HMPA polyester dendron blocks and PEG were synthesized using the Diels–Alder "click" cycloaddition reaction by Gok et al.(36)

Kempe et al. reported the synthesis, in a one-pot cascade reaction approach, of a bis-HMPA dendron functionalized at the focal point with poly(2-ethyl-2-oxazoline) (Pox). Pox is a polymer with similar properties to PEG, such as protein repellency and stealth behavior; thus, it is being extensively discussed as a potential alternative to the use of PEG.(37)

Recently, Feliu et al. carried out a comprehensive assessment of the in vitro biocompatibility and degradability of a library of hydroxyl terminated bis-HMPA dendrimers of five different generations and their corresponding dendrons, that revealed excellent biocompatibility for these materials in the model systems used.(38) Besides the expected pH dependence of the degradation rate, in line with the previously reported data for bis-HMPA dendronized PCL,(31) it was found that the hydrolysis occurs first at the dendrimer periphery and progresses toward the core, through a mechanism of depolymerization. Additionally, sterically crowed G4-OH dendrimers were found to be extensively degraded after 2 days of incubation at pH 7.5.(38)

Thus, despite the widespread use of these bis-HMPA-based dendritic structures, some issues regarding premature or undesired degradation still remain to be addressed. Namely, the unwanted degradation of the PEGylated polyester backbone observed during the attachment of certain drugs.(39) Also, a significant degradation after only 10 h under physiological conditions (pH 7.4 and 37 °C) was observed by van der Poll et al. for a G1 bis-HMPA-core dendrimer, which limits their potential use in certain biological applications.(16a) In order to solve this problem, these authors reported a synthesis that combines the biocompatibility and biodegradability of polyester dendrimers with the robustness of polyamide dendrimers, to yield a hybrid scaffold capable of translation into in vivo studies. In this manner, the degradation rate was significantly reduced, with the degradation profile for these ester–amide hybrid dendrimers being prolonged up to 20 days.(16a).

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3.2 Other Aliphatic Ester Monomers

Other aliphatic ester monomers have been used to synthesize polyester dendrimers, although to a smaller extent than bis-HMPA. Carnahan and Grinstaff have reported polyester dendrimers coined as "biodendrimers", which are composed of natural metabolites and biocompatible with the human body. These authors developed several dendrimers based on only succinic acid, only adipic acid, and mixtures of the two (Figure 3a).(40) The properties of the mixed dendrimers depends on the composition of the outer generation layer.(40c) The esterification of the dendrimers with succinic acid monomethylallyl ether, and subsequent photochemical polymerization of the alkenes, yielded soft gels.(40a) These materials have been explored as corneal adhesives(41) and for cartilage repair.(42).

The synthesis of another family of biodegradable cationic dendrimers was proposed in 2006 (Figure 3b).(43) The ester-amine dendrons and dendrimers synthesized in this work by the convergent method up to G3 and G2, respectively, were based on bis(2-hydroxy-ethyl)-amino]-acetic acid tert-butyl ester as the growing unit (Figure 3b), with internal tertiary amines and protected terminal amines. The synthesized dendrons and dendrimers, with their terminal amines deprotected, were degradable in D_2O at 37 °C. The degradation rates were found to be dependent on the generation number and the ability of the primary amines to access and to catalyze the ester group degradation. All dendrons were completely degraded within 30 days, while only 60% of the sterically crowded dendrimers were degraded over the same time period.(43)

Twibanire et al. synthesized tribranched dendrons in order to prepare polyester dendrimers with denser layers than those derived from bis-HMPA and, therefore, more resistant under physiological conditions.(21g) The synthesis of polyester dendrimers using as starting materials benzyl acetoacetate and tert-butyl acrylate (Figure 3c) were presented by Hirayama et al.(44) Similar dendrimers, also designed to be applied as drug delivery vectors, were prepared from 3-hydroxyacetophenone and its tert-butyldimethylsilyl ether.(45)

Polyester dendrimers containing tertiary amines were prepared by Bouillon et al.(46) The amine groups can serve as buffers to neutralize the protons delivered from the ester hydrolysis; therefore, these poly(ester amine) dendrimers are very attractive as drug delivery vectors (Figure 3d).

Another interesting contribution was the synthesis of polyester dendrimers presenting functional groups capable of orthogonal reactions,(47) rendering bifunctional dendrimers.(48) An AB_2C dendron (Figure 3e.1) was used to originate dendrimers bearing alkyne units and hydroxyl groups (Figure 3e.2). A bifunctional dendrimer hosting azide and alcohol functionalities was also synthesized.(48)

In 2013, Akiyama et al. presented divergent and convergent procedures for the synthesis of another type of poly(ester amine) dendrimers, having an adamantane structure as core, which allows that even low generation dendrimers have a globular structure.(49) Recently, Pahovnik and et al. also reported on the synthesis of different generation, water-soluble poly(ester amide) dendrimers with hydroxyl functional groups from an AB_2 adduct of bis-HMPA and glycine as repeating unit and 1,1,1-tris(hydroxymethyl)propane as core. These poly(ester amide) dendrimers were used for solubilizing an anti-diabetic drug.(50)

3.3 Alternating Polyester Dendrimers

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Different types of dendrimers, called by some authors "alternating dendrimers",(51) can be produced in which the ester linkages alternate with other types of linkages, if orthogonal coupling methods are used in alternation.(48, 51, 52) The alternation of click reactions with ester formation using two different AB2 dendrons (Figure 4a.1) for the accelerated synthesis of the dendrimers was reported by Antoni et al.(53) Because these orthogonal reactions do not require protecting groups and, thus, activation or deprotection steps are not needed, only five steps led to the preparation of a G4 dendrimer (Figure 4a.2).

Ma et al. developed a facile synthesis of biodegradable alternating polyester dendrimers from sequential click coupling of asymmetrical monomers (2-[(methacryloyl)oxy]ethyl acrylate (MAEA) and cysteamine), with pendant methacrylate or amine groups that can be easily used for conjugations of drugs.(54) Later, Ye et al. simplified the synthesis by using a β-cyclodextrin core, from which polyester dendrimers with high molecular weights could be easily synthesized in fewer steps without intensive purifications.(14) Moreover, they carried out the in vivo evaluation of these biodegradable dendrimers as MRI contrast agents, the data of which will be further discussed in section 6.(14) Montañez et al. used AB2 dendrons for the development of another approach for the accelerated synthesis of polyester dendrimers with terminal alkene bonds.(55) Walter et al. developed a series of macrothiols bearing latent hydroxyls (Figure 4b.1).(56) Dendrimers based on bis-HMPA were obtained through the photochemically induced addition of these macrothiols to alkene-terminated core molecules. Deprotection of the latent hydroxyls originated a hydroxyl-terminated dendrimer (Figure 4b.2) that can then be functionalizated further to give products with desired properties.(56)

An efficient convergent synthetic route alternating esterification with light-promoted addition of a thiol to an alkyne was described by Chen et al. This dendrimer was shown to effectively bind the anticancer drug cis-dichlorodiammineplatinum(II).(57) Rosen et al. presented another alternating synthetic polyester dendrimers route for synthesizing dendrimers containing α-bromo esters, which can polymerize by a single electron transfer living radical polymerization and thus produce star polymers with low generations of dendrimer units at the center and on the periphery.(58) Downing et al. reported that acyloxysilyl bonds (silyl esters) can be incorporated into dendritic structures as an easily degradable bond.(59)

In 2013, Khoee et al. published the preparation of pH-susceptible linear–dendritic–linear block copolymers from poly(ε-caprolactone), asymmetric poly(ester amine) dendrons (G1–G3), and PEG.(60) Their hydrolytic degradation was investigated at two pHs (5.8 and 7.4) at 37 °C. They observed that the copolymers with the higher-generation dendrons degraded more slowly than those with lower generations. Furthermore, in this case, the degradation rate at pH 5.8 is faster than at pH 7.4 for all generations. These amphiphilic systems can self-assemble and form micelles in water. Thus, they designed nanoparticles containing magnetite coated with these block copolymers for encapsulating hydrophobic drugs, such as quercetin. Their sensitivity at lower pHs is an attractive feature for using these nanoparticles for drug delivery in vivo.(60)

3.4 Degradable Dendrimers Not Based on Ester Bonds

An approach to obtain polyacetal systems using sequential transacetalation and protection–deprotection techniques has been proposed by Fuchs and Lemcoff for the preparation of macromolecular polyacetals that were subsequently applied to obtain new dendrimers, which may undergo hydrolysis to effectively "unzip" the dendrimer to polyfunctional macromolecules or degrade it altogether.(61)

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Using an iterative and divergent approach, Zimmerman and co-workers synthesized dendrimers from degradable 1,3,5-triazaadamantanes (TAA) monomers at each branch point (Figure 4c).(62) Contrary to some polyester dendrimers, TAAs are stable under basic and physiological conditions (pH 7.4), but hydrolyze rapidly (half-life lower than 30 min) under acidic conditions (e.g., endosomal pH) to give basic and well-defined byproducts.(62a)

In the following sections, the applications of biodegradable dendritic structures as vectors of both drugs and nucleic acids, as well as their function as macromolecular contrast agents in MRI, are revisited.

4 Biodegradable Dendritic Structures in Drug Delivery

One of the first applications of dendrimers in the biomedical field was as drug delivery vectors, since they can easily transport drug molecules in their interior and/or tethered to their terminal surface groups.(5a, 63)

The advantages of using dendrimers as drug carriers are multiple and include the following: (a) the possibility of being designed to carry hydrophobic or hydrophilic drugs, protecting them from degradative processes or unwanted interactions with biological molecules; (b) the capacity to simultaneously release two or more drugs at the site of interest allowing the tuning of pharmacodynamics; (c) the potential to modify the dendrimer surface to confer hydrophilicity to the dendrimers, to help them to surpass important biological barriers (such as those imposed by the mononuclear phagocyte system or cellular membranes), or to endow them with chemical ligands that are recognized by cell receptors and actively target specific types of cells or tissues; (d) the chance to play with the dendrimer size (generation) to modulate the dendrimer's blood circulating time and/or passively target solid tumors through the enhanced permeability and retention (EPR) effect; (e) the ability to achieve a controlled delivery of the drugs by controlling the dendrimer/drug interactions or by developing stimuli-responsive systems; and (f) the possibility to use dendrimers as theranostic platforms through their combination with chemical entities/nanoparticles suitable as contrast agents in bioimaging (this point will be further explored in section 6).(5a, 63)

Biodegradable dendrimers show all the mentioned potentialities of dendrimers for drug delivery, further presenting the advantage of being transformed under physiological conditions into small-size products that may be metabolized or excreted from the body. In this context, the dendrimers and their products of degradation should show a low level of toxicity, as is the case for polyester dendrimers.(19, 38, 64) About 12 years ago, in a representative work, Padilla De Jesús evaluated the toxicity both in vitro and in vivo of several dendritic structures based on bis-HMPA.(64) All the structures were shown to have a low cytotoxicity and a high tolerability upon administration in mice by intravenous (i.v.) bolus injection. One of these structures, a high-molecular-weight 3-arm PEG-dendrimer hybrid (that exhibited the longest circulatory half-life) was conjugated to doxorubicin by means of an acid-labile hydrazone linkage. In vitro experiments showed that the system was able to release the drug as a function of pH with a higher release at pH below 6. Results also indicated that the cytotoxicity of the drug was significantly reduced after linking it to the dendrimer, demonstrating that the use of a carrier alters the pharmacokinetics and the distribution of the drug. The design of the dendrimer structure to prevent rapid and premature degradation was also revealed as an important feature toward obtaining an effective drug delivery system. Later, the same group reported a study where orthogonal "bow-tie" polyester dendrimers were prepared with PEG arms connected to one of the polyester dendrons via degradable carbamate bonds (Figure 2d).(19) Here, the polyester dendron was conjugated with the

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drug and, while complete ester hydrolysis alone should release the bis-HMPA monomers, carbamate hydrolysis should release the individual PEG arms. A library of eight polyester dendrimer-PEG bow-tie hybrids was prepared (molecular weights from 20 000 to 160 000, and number of PEG arms ranging from two to eight) and evaluated as controlled drug release systems. In this work, as expected, the high-molecular-weight carriers (>40 000) exhibited longer circulation half-lifes. Furthermore, the in vitro degradability of the G3 bow-tie with 10 kDa PEG was followed for 15 days at pH 9.0, pH 7.4, and pH 5.0 and 37 °C. The reported results were in line with the ones reported by Lee et al. for the bis-HMPA dendronized PCL.(31) Additionally, the obtained results showed that at pH 5.0 only the carbamate bonds degrade, while at pH 7.4 the ester bond present in the bis-HMPA dendron backbone also degrades.(19) A subsequent study showed the effectiveness of these carriers as drug vehicles.(65) Indeed, a single injection of a high-molecular-weight dendrimer–PEG–doxorubicin conjugate substantially inhibited the progression of a doxorubicin-insensitive C-26 colon carcinoma upon i.v. administration to BALB/c mice. A PEGylated dendrimer based on a polyester-polyamide hybrid backbone was also described for doxorubicin transport through conjugation.(16a) As previously mentioned at the end of section 3.1, the design of the carrier was chosen to diminish destructive side reactions during dendrimer synthesis and premature degradation, while a suitable biodegradability is equally maintained. The anticancer efficiency of the dendrimer–doxorubicin conjugate was compared to that of Doxil using equal dosages in the treatment of C26 murine colon carcinoma. In this work, statistically equivalent results were obtained with the two systems with most mice tumor-free at the end of the two-month experiment, with the test group animals indicating that the ester–amide dendrimer may exhibit less toxicity than Doxil at equivalent doxorubicin dosages.(16a)

The encapsulation of drugs inside dendritic structures is a common strategy in drug delivery that can help in the solubilization of hydrophobic drugs such as the camptothecins that have low water solubility but high anticancer efficiency. Morgan et al. used one of their "biodendrimers", the one based on glycerol and succinic acid, for the encapsulation of 10-hydroxycamptothecin and 7-butyl-10-aminocamptothecin.(66) The cytotoxicity of the dendrimer–drug complexes toward four different human cancer cell lines was analyzed showing low IC50 values. Importantly, cellular uptake and drug retention were increased in MCF-7 cells when using the prepared dendrimer.(66) In another study, Dhanikula and Hildgen investigated the influence of the architecture of polyester-co-polyether (PEPE) dendrimers on the encapsulation and release of the anticancer drug methotrexate.(67) In vitro experiments showed that the different dendrimers were biocompatible and presented a good capacity to encapsulate this drug. An increase in the number of branches and in the size of internal cavities was shown to enhance the encapsulation capacity, while the absence of aromatic rings as branching units substantially decreased the loading capacity of PEPE dendrimers. The authors concluded that the mechanisms of encapsulation involved physical entrapment, weak hydrogen bonding, and hydrophobic interactions.(67)

Interestingly, thermoreversible hydrogels were prepared by Namazi and Adeli from dendronized polymers based on citric acid (dendron part) and PEG (linear part).(68) It should be highlighted that several hydrophobic anti-inflammatory drugs have been successfully encapsulated inside the formed gels. Lundberg et al. presented bis-HMPA dendrons of different generations (G0–G4) as functionalized macroinitiators for the construction of sophisticated amphiphilic dendritic-linear hybrid materials with hydrophobic poly(ε-caprolactone) at the chain-ends and a single strain of hydrophilic PEG at the core.(69) These were utilized for the fabrication of drug loaded micelles as well as the development of isoporous membranes.

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Though still at a preliminary stage, "disassembled", "cleavable", or "self-immolative" dendrimers (SIDs) that respond to an external stimulus (e.g., pH variation, enzymatic action, catalytic antibodies, among others) constitute very promising systems for drug delivery.(70) These dendrimers have a unique structural degradable backbone, the most common cleavage functionalities of which are esters and carbamates, that allows a cascade decomposition upon a simple triggering event. If drug molecules are incorporated as the tail units in these dendrimers and a suitable stimulus is used as the trigger, a multi-prodrug is generated that may be activated by a single cleavage event. The advantages associated with these dendritic prodrugs are significant when compared with monomeric prodrugs in inhibition of tumor cell growth.(71)

Biodegradable dendrimers have also been used in the delivery of boron-10 for neutron capture therapy (NCT) of cancer. For this, it is crucial to ensure an adequate boron concentration at the target sites, and as such, high boron content species (like carboranes) can be used in the design of the carriers. The polyester dendrimers based on bis-HMPA proposed by Parrott et al. incorporated several carboranes within its structure (section 3.1).(21m) Fourth- and fifth-generation dendrimers that contained 4, 8, and 16 carborane cages were prepared. The irradiation of these dendrimers with thermal neutrons resulted in emission of γ radiation, thus showing the occurrence of boron neutron capture events and the potentiality of using these dendrimers in cancer treatment.(21m)

5 Biodegradable Dendritic Structures for Nucleic Acid Delivery

Over the past decades, gene therapy has emerged as a promising therapeutic approach to prevent and treat several diseases/conditions. Its underlying principles are the modulation of gene and/or protein expression of the host cells following the introduction of exogenous nucleic acids (DNA, RNA, or single-stranded oligonucleotides) into somatic cells.(72)

Nucleic acids (NAs) are susceptible to degradation by serum endonucleases, further decreasing their already low internalization when administered naked.(1e) Moreover, following cellular uptake, NAs are susceptible to additional degradation both in the lysosomal/endosomal pathway and in the cytoplasm.(72, 73) Thus, most of the gene therapy strategies proposed so far rely on delivery vectors that, ideally, should contribute to the overcoming of different extra- and intracellular barriers in order to efficiently deliver NAs into cells with minimal toxicity.(72, 73) Briefly, the main hurdles toward a successful gene therapeutic intervention include the following: (a) NA degradation by endonucleases present in the extracellular milieu, (b) cellular internalization, (c) endosomal escape, (d) NA release from the vector and access to the cytoplasmic or nuclear target, and (e) vector intra- and extracellular accumulation. Nuclear delivery, necessary in the case of DNA(72, 73) or certain oligonucleotides,(74) further requires that the NA crosses the nuclear membrane. Additionally, the delivery vector must avoid unspecific binding to serum proteins, preventing aggregation.(5c, 72a, 73) NA vectors are divided into two main categories: viral and nonviral vectors.(72a, 73) Despite the high transfection rates observed for viral vectors, insertional mutagenesis and immunogenicity together with both low scale production and carrying capacity are delaying their application as safe and viable gene therapy vectors.(72a, 73, 75) Consequently, these obstacles have drawn much attention to the development of nonviral vectors, such as lipids, polymers, and dendrimers.(1e, 72a, 73) A common feature among these has been their cationic nature. The same characteristics that make dendrimers attractive platforms for drug delivery are equally important when it comes to the delivery of therapeutic NAs. Their adaptable and tunable chemistry together with the high density of functional groups allows the design of an almost unlimited number of molecules and the conjugation of several ligands tailored to attain efficient

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gene delivery.(5c, 63a) Nevertheless, much research is needed for these to progress into preclinical and clinical developments.

Cationic dendrimers are able to complex NAs through electrostatic interactions between their positively charged terminal groups and the negatively charged phosphate groups of the NA, originating dendriplexes.(5c, 72a) This process occurs in a concentration-dependent manner and is also affected by the medium properties, such as pH, temperature, and ionic concentration.(5c)

Even though several groups reported the success of dendrimers as NA delivery vehicles,(7b, 7h, 76) the nonbiodegradability of the dendritic structures used remains a drawback yet to be solved.(6a, 8d, 63a) As previously mentioned, the ideal gene delivery vehicle should be biodegradable to prevent bioaccumulation and subsequent cytotoxicity.(8) Moreover, biodegradability can contribute to the carrier's multivalency decreasing as a function of time, leading to a lower interaction with the transported NA, promoting its release.(77)

So far, the few reports in the open literature on the use of biodegradable dendritic structures for gene delivery have been based on the application of bis-HMPA-based dendrons. Welsh et al. synthesized bis-HMPA based dendrons up to the third generation (G3) with carbamate-linked spermine groups on their surface and a benzyl ester protecting group at the focal point.(77a) The efficiency of DNA binding increased in a generation dependent manner due to the enhanced multivalency. Degradation studies showed that the G3 dendrimer was stable at pH 5.0, but degraded at the physiological pH 7.4 on the time scale of 8 days.(77a) In a slightly different approach, Barnard et al. replaced the benzyl ester protecting group in G2 dendrons by hydrophobic units (cholesterol and hydrocarbon chains) to promote their controlled self-assembly rendering a system with higher multivalency, which significantly enhances the DNA binding. Furthermore, they modified the surface groups with N,N-di(3-aminopropyl)-N-(methyl)amine (DAPMA), a triamine that showed lower cytoxicity than spermine.(77b) As expected, these dendrons remained intact at pH 5.0 and degraded at pH 7.5. Even though cholesterol-functionalized dendrons were shown to have lower degradation rates than the hydrocarbon-functionalized ones, every dendron was completely degraded after a period of 6–10 h.(77b) The reported G2 dendrons could efficiently complex DNA and undergo cellular internalization, yet a low transfection efficiency was observed. The authors hypothesized that, at the lower endosomal pH, or when bound to DNA, the degradation of these dendrons becomes ineffective on the transfection time scale, which could explain their poor transfection performance.(77b) The same group further modified the cholesterol-functionalized G2 dendrons with small PEG chains (triethylene glycol and octaethylene glycol).(78) PEG addition was found to affect size and zeta potential properties of the resulting dendriplexes and enhanced DNA binding to different extents, with a higher binding affinity observed for the structures based on the longer PEG chain.

More recently, Barnard and et al. have reported a different strategy aimed at improving DNA release and dendron degradation.(79) They synthesized self-assembly disulfide-linked dendron nanoparticles, where the cholesterol groups were attached to the DAPMA-terminated G2 bis-HMPA dendrons by an S–S linkage. In vitro, upon addition of dithiothreitol, nanoparticles where shown to undergo disassembly in 1 h due to cleavage of the disulfide linkage, which consequently led to loss of self-assembled multivalent binding, triggering DNA release (Figure 5). After that, the remaining fragments undergo further degradation over longer time scales (24 h), which validates a controllable double-degradation process.(79) Disulfide linkers are widely used in the design of nonviral gene delivery vectors due to their enhanced degradability in the reductive environment of the cytoplasm.(72)

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The success of gene therapy is highly dependent on the development of efficient and safe NA delivery vectors. Additionally, it is extremely important to develop new biodegradable and biocompatible vectors to prevent both bioaccumulation and cytotoxicity, and easy clinical translation. Such vectors could arise from newly developed dendritic agents, which allow an almost unlimited combination of structures and architectures together with the ability to multiconjugate several target-molecules.

6 Biodegradable Dendritic Structures as Magnetic Resonance Imaging Contrast Agents

Currently, medical imaging plays a major role in health care management as one of the most efficient techniques for early and accurate diagnosis and treatment follow-up, expectedly leading to more successful treatment. As a noninvasive technique, MRI allows high spatial resolution and both physiological and anatomical information without resorting to harmful ionizing radiation. However, both its low sensitivity and target-specificity remain significant limitations.(5d)

MRI is based on the interaction of protons (mostly water protons) with each other and their surroundings. Upon the application of strong magnetic fields, the magnetic moments of protons will orient themselves in the direction of the magnetic field.(80) After disruption of this magnetic alignment they will return to the previous state in a process described by two relaxation rates: the longitudinal and the transversal relaxations, characterized by the time constants T_1 and T_2, respectively.(80, 81) The relaxation rates vary from tissue to tissue, which gives rise to the image contrast in MRI. However, the inherent differences in relaxation times among tissues are not always enough to generate an adequate contrast. Thus, it is rather common to use exogenous contrast agents (CA), which act by reducing T_1 and T_2 through interaction with the surrounding water protons.(5d, 80, 81) The ability to decrease T_1 and T_2 is measured by the r_1 and r_2 relaxivities, respectively ($r_1 = 1/T_1$; $r_2 = 1/T_2$).(80, 81)

Contrast agents can be classified by their biodistribution, magnetic properties (paramagnetic and superparamagnetic), and effect on the image contrast, which can be positive (T_1 reduction) or negative (T_2 reduction), increasing or decreasing the signal intensity, respectively.(82) Paramagnetic agents are predominantly T_1-reducing CA, while superparamagnetic usually originate T_2 reductions.(82) The most widely used CAs are small molecules based on paramagnetic gadolinium(III) chelates, such as Gd(III)-DTPA (DTPA = diethylenetriaminepentaacetic acid, Magnevist) and Gd(III)-DOTA (Dotarem).(5d, 83) Administered prior to the MR imaging, these CAs are known for their contrast enhancement. Yet, their low molecular relaxivity, nonspecificity, and fast renal clearance require the usage of potentially harmful high doses, which clearly limits image quality.(5d) Conjugation of Gd(III)-chelates with polymers,(84) proteins,(85) dendrimers,(5d) or other scaffolds(86) can enhance relaxivity and blood circulation times improving image contrast.(1f)

Due to their multivalency, dendrimers represent one of the most appealing platforms to carry multiple CAs moieties.(5d) Moreover, their branched structure brings an extra rigidity and the possibility to graft target molecules, offering a unique opportunity to enhance site-specific image contrast (Figure 6).(81)

The applicability of dendrimers as MRI contrast agents (dendritic CAs, DCAs) was first demonstrated by Wiener et al. where a PAMAM dendrimer conjugated with a Gd(III)-DPTA showed an ionic relaxivity of 34 mM–1 s–1, meaning a 6-fold higher relaxivity compared to that of Gd(III)-DTPA alone (5.4 mM–1 s–1).(87) Additionally, they observed that these novel structures were able to carry more Gd(III) ions than other existing macromolecules at the time, which could possibly be increased at higher generations. Although the macromolecular CAs developed so far have been yielding good image

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contrast and enhanced sensitivity, the large sizes decrease renal excretion, which in turn significantly increases the possibility of bioaccumulation and further release of toxic Gd(III) ions.(5d, 83, 88) In fact, some studies have reported that nephrogenic fibrosis and other severe diseases were associated with the use of certain Gd(III) chelates, particularly in patients with impaired renal function.(89) So far, only Gadomer-17, a poly(lysine)-based dendritic CA bearing 24 Gd(III)-DOTA chelates, has advanced into clinical evaluation due to its almost exclusive intravascular biodistribution and rapid renal clearance.(90)

Thus, recent efforts have been made to develop biocompatible and biodegradable DCAs (Table 1). These maintain the nondegradable DCAs characteristics, such as blood circulation times and enhanced image contrast, and at the same time can be easily degraded under physiological conditions into smaller byproducts preventing accumulation and subsequent toxicity.

Nazemi et al. designed a dendritic Gd(III)-DTPA functionalized polymersome.(91) The azide-terminated polymersome consisted of a PCL–PEG block copolymer and a G3 bis-HMPA polyester (PE) dendron. Ionic relaxivities (r1) of 12.1 and 26.1 mM–1 s–1 (20 MHz and 25 °C) were observed for the dendron alone and the dendron-functionalized polymersomes, respectively.(91) While most of the DCAs developed so far have been targeted to r1 augmentation, Klemm et al. focused on designing contrast agents to improve r2.(92, 94)T1 CAs usually lose efficiency at higher magnetic fields, which constitutes an important limitation since clinical instruments work at increased magnetic fields to enhance image contrast. In contrast, T2 CAs exhibit higher relaxivities at higher magnetic fields.(92, 94) As reported by Klemm et al., a versatile CA with the ability to enhance r1 and r2 would bring an excellent opportunity to improve both imaging modes.(92) In this sense, they conjugated a Gd(III)–TACN–bis(1-Me)–3,2-HOPO–TAM–ethylamine to an ester–amide (EA) dendrimer via an amide bond.(92) Remarkably, these complexes were able to increase Gd(III) r1 and r2 up to 31 and 52 mM–1 s–1, respectively. This represents a 10-fold increase in r1 compared to that of the clinically available CAs. Moreover, this DCA was shown to be nontoxic at concentrations up to 25 μM at 72 h.(92) Other studies published by the same group described similar DCAs conjugated with other chelating molecules that enhanced r1 and r2, as well.(94, 95) As mentioned in section 3.3, in 2012, Ma et al. synthesized Go to G3 alternating polyester dendrimers, based on MAEA and cysteamine monomers with a β-cyclodextrin core.(54) This dendrimer was further conjugated with Gd(III)-DTPA and evaluated in vivo as DCA.(14) Go had a relaxivity of 10.6 mM–1 s–1, while G1, G2, and G3 showed similar relaxivity values around 11.7 mM–1 s–1. This phenomenon was attributed to the comparable internal flexibility of the higher generations, which hindered the expected rise of the relaxivity with the generation. Again, degradation studies, using the G3 dendrimer, showed that at mildly acidic pH little or no degradation was detected, while at pH 7.4 only 70% of the ester bonds remained intact 20 h post-incubation. This percentage decreased to 35% in the presence of an esterase. For in vivo studies the authors introduced zwitterions into the dendrimer to enhance solubility. The zwitterionic G2 DCA was shown to have higher blood circulation times and image improvement than the clinically used Magnevist. Additionally, this dendrimer was shown to promote lower long-term Gd(III) accumulation in several tissues when compared to the nondegradable dendritic MRI agent PAMAM-G6-DO3A-Gd(III) (DO3A = 1,4,7-tricarboxymethyl-1,4,7,10-tetraazacyclododecane).(14)

More recently, molecular MRI has also drawn attention due to its potential use in the dissection of disease mechanisms and other events at the cellular and subcellular levels, which can be achieved by combining CAs with target molecules.(5d, 96) In 2012, the G2 polyester dendrimer reported above was further functionalized with PEG and folic acid (FA) to target tumor cells overexpressing folic acid receptors.(14, 89a) PEGylation resulted in enhanced relaxivity due to the increasing solubility and

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complex stiffness. FA-PEG-G2-DTPA-Gd(III) had a higher and longer tumor image contrast than that of Magnevist and a much lower Gd(III) accumulation compared with nondegradable contrast agents, due to dendrimer degradation. Degradation studies showed that 30% of the ester bonds of this DCA hydrolyzed at pH 7.4 against 7% at pH 5.0 after 10 h. The hydrolyzed ester bonds at pH 7.4 were shown to increase to 47% in the presence of an esterase.(89a) Despite the great performance of this DCA, the need for solubilizing groups might hamper translation to the clinic.(93) In order to overcome this, the same team developed a new DCA based on the same polyester dendrimer, but with a tris(2-aminoethyl)amine core to increase solubility without further modifications (PEGylation), hence preventing high molecular weights and size distribution.(93) As expected, they observed that r1 increased from 10.2 to 17.5 mM–1 s–1 when going from G1 to G5 dendrimer, demonstrating a generation-dependent relaxivity. Interestingly, in vivo MRI revealed major biodistribution differences between the G2 and G5 dendrimers, as the first enhanced tumor contrast whereas the second enhanced liver signal. The smaller size of the G2 allowed improved tumor imaging due to the passive tumor targeting via the EPR effect.(93, 97) As expected, G2-DTPA-Gd(III) displayed pH-dependent degradability, being stable at pH 5.0 and degraded at pH 7.4 (20 h post-incubation, 80% of the ester bonds were degraded).(93)

Biodegradable dendritic-based MRI contrast agents have already proven to be a promising alternative to the currently available CAs. Even though the significant molecular weight increase could be a limitation to the clinical setting, it remains true that lower amounts of CA would be necessary due to the relaxivity increase. Therefore, further testing is needed to formulate an adequate molecular weight/relaxivity ratio. Success could rely on the optimization of already existing systems, as well as on the development of new biodegradable and biocompatible dendritic platforms. Moreover, the combination of several MRI labels and target-specific molecules in the same dendritic scaffold could give rise to better and more accurate diagnostics.

7 Conclusions and Future Perspectives

The use of degradable polymeric systems has a wide perspective in several established and emerging biomedical technologies, including controlled-release systems for targeted drug delivery, antibacterial drugs, and tissue regeneration. As the demand increases, a higher level of control over the structure, properties, and development of degradable materials is being pursued.

Dendrimers allow this coveted structural control. Among these, biodegradable dendrimers represent an attractive class of nanomaterials. They possess two major advantages over the conventional dendrimers, which confers an additional inherent "smartness" to these systems: (a) multiple covalently bound bioactive molecules can be site-specifically released from the targeted dendrimer by a single cleaving step, and (b) they are degraded and therefore can be easily eliminated from the body avoiding the toxicity related to the accumulation of synthetic materials in cells/tissues. Consequently, biodegradable dendrimers are expected to surpass the limitations of the most used nondegradable systems. Even so, the design and synthesis of biodegradable dendritic structures soluble in water with precise branching, molecular weight, monodispersion, and with a suitable pharmacodynamics continues to be a challenge. Other important key aspects are to overcome the undesired degradation of the degradable backbone observed during the attachment of certain functional groups and/or bioactives, as well as to limit the premature hydrolysis of the backbone and/or functional moieties in order to ensure their efficient application. In fact, these hurdles may explain the reduced number of reports where biodegradable dendrimers are applied for a particular function, in comparison with the higher number of works reporting only the design and synthesis of biodegradable dendrimers. This will

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require considerable effort in the development of new synthetic routes for the development of these systems.

Nevertheless, once these drawbacks are surpassed, biodegradable dendrimers are expected to recast the existing therapeutic practices. The simultaneous combination of multivalency and biodegradability with precise architectures will definitely make dendrimers a greater platform, versatile for many biomedical applications as revised here.

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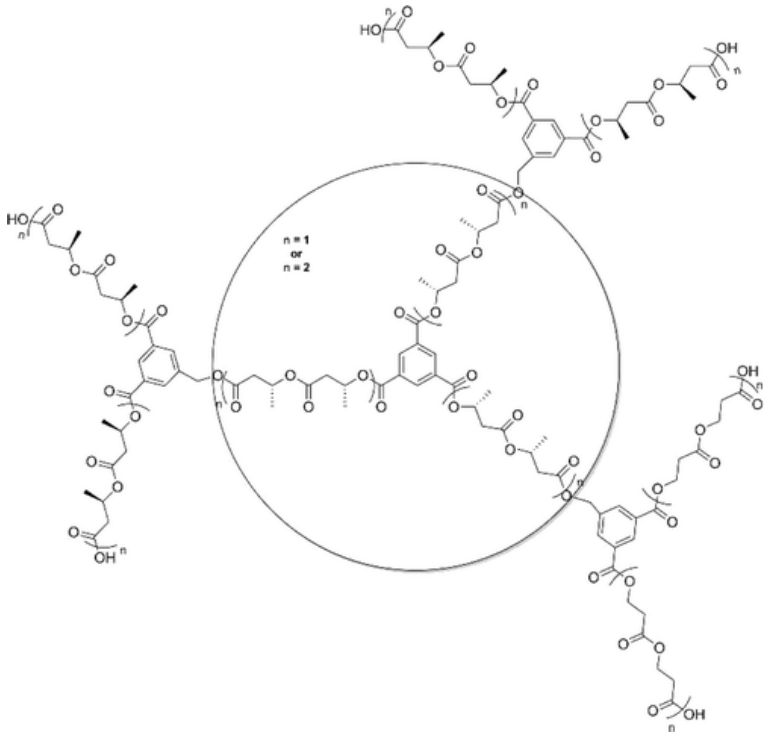

Figure 1. Generation 1 (G1) of the first published biodegradable dendrimer (the dendrimer core is depicted within the circle).

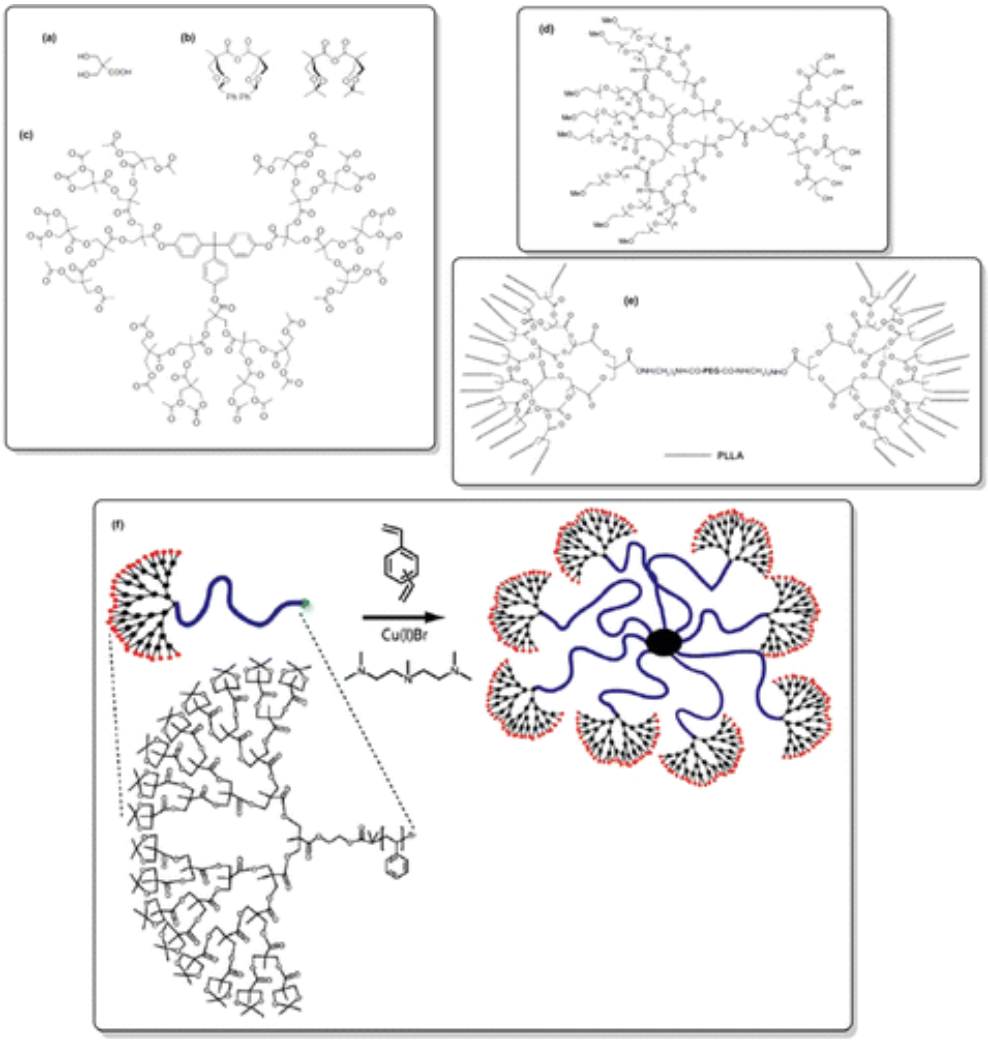

Figure 2. Bis-HMPA based dendrimers (a) 2,2-bis(hydroxymethyl)propanoic acid (bis-HMPA). (b) Protected anhydrides for the synthesis of bis-HMPA based dendrimers. (c) Generation 3 (G3) of the first synthesized bis-HMPA dendrimer. (d) "Bow-tie" dendrimer. (e) Structure of dumbbell-shaped triblock copolymer (adapted from Biodegradable comb–dendritic triblock copolymers consisting of poly(ethylene glycol) and poly(l-lactide): Synthesis, characterizations, and regulation of surface morphology and cell responses, Gong, F., Cheng, X., Wang, S., Wang, Y., Gao, Y., Cheng, S. Polymer 2009, 50, 2775–2785 reprinted with permission from Elsevier). (f) Synthesis of Generation 5 functionalized Core Cross-linked Star (CCS) polymers via the "arm first" approach. (Reproduced from Macromolecules2007, 40, 7855–7863 with permission from The Royal Society of Chemistry).

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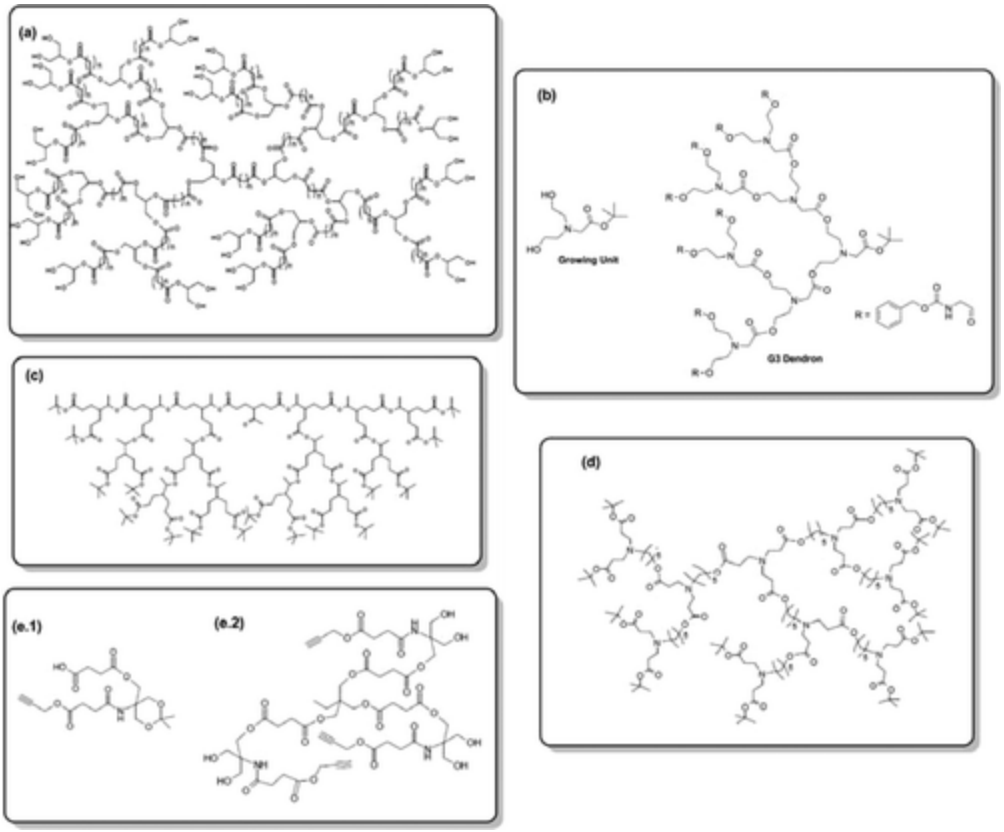

Figure 3. (a) Generation 3 (G_3) of a polyester "biodendrimer". (b) Growing unit and Generation 3 (G_3) of biodegradable ester-amine dendrons. (c) Generation 3 (G_3) of the acetoacetate tert-butyl acrylate derived dendrimer. (d) Second generation (G_2) of amine-containing polyester dendrimer. (e) Bifunctional dendrimers: (e.1) AB_2C dendron, (e.2) first generation (G_1) of the bifunctional dendrimer with three alkynes and six hydroxyls.

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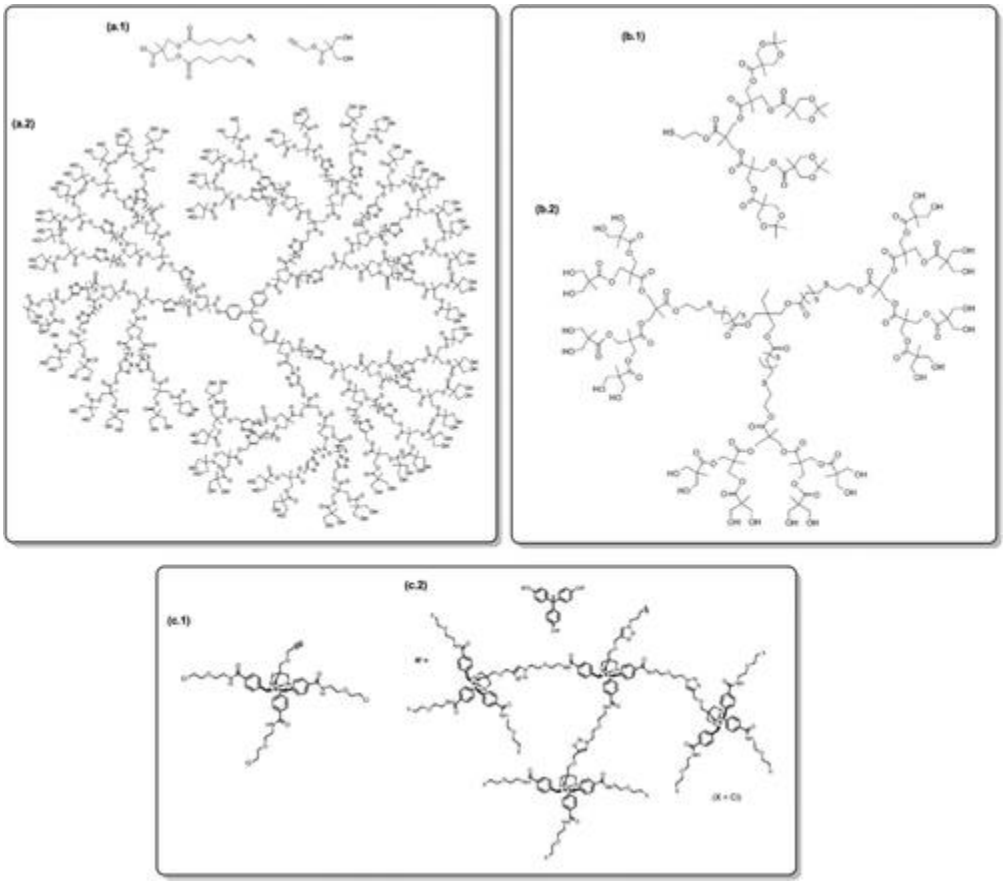

Figure 4. (a) Alternating polyester dendrimer: (a.1) AB2 dendrons, (a.2) fourth generation (G4) dendrimer resulting from accelerated synthesis. (b) Alternating polyester dendrimer: (b.1) macrothiol-dendron bearing latent hydroxyls, (b.2) hydroxyl-terminated dendrimer based on macrothiol-dendrons. (c) Degradable TAA-based dendrimers: (c.1) degradable TAA monomer, (c.2) generation 2 (G2) of TAA-based dendrimers.

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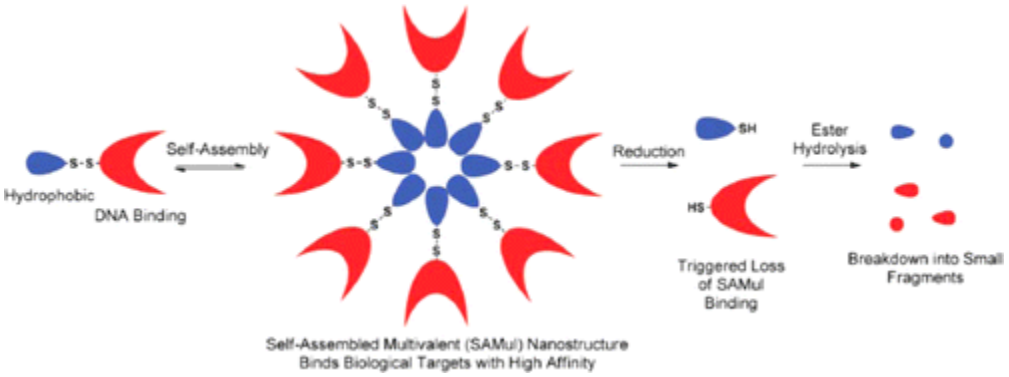

Figure 5. Schematic representation of the concept of triggered loss of multivalent binding (reproduced from Org. Biomol. Chem.2014, 12, 446–455 with permission of The Royal Society of Chemistry).

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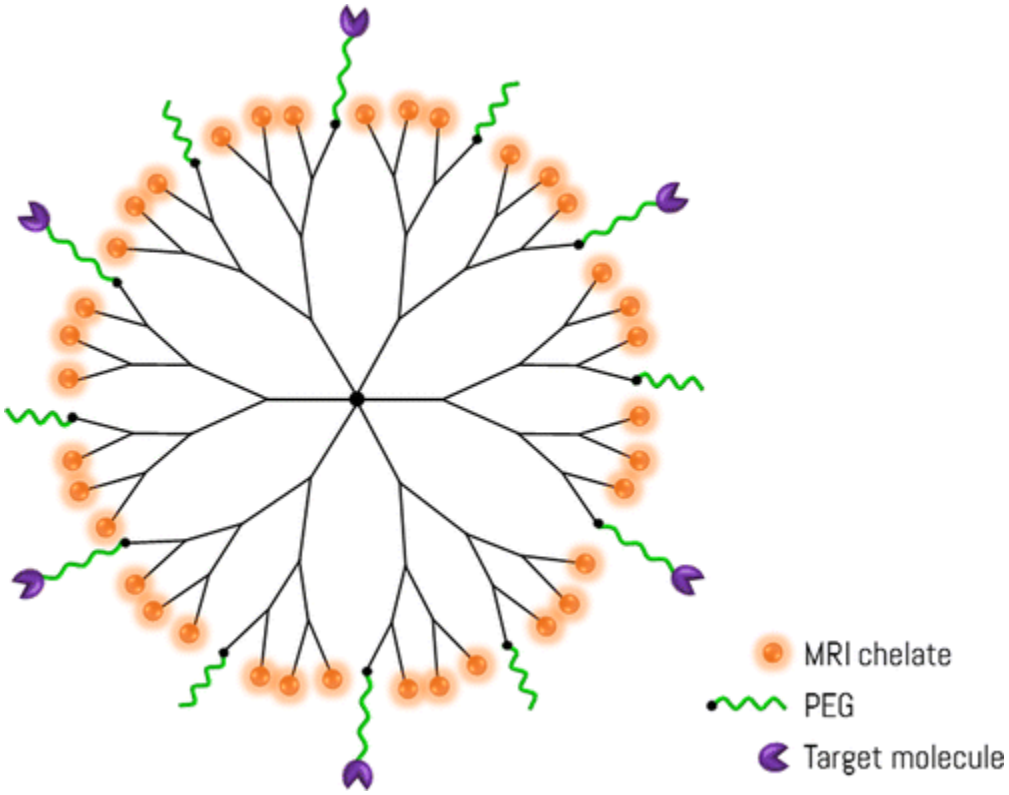

Figure 6. Schematic representation of a PEGylated and targeted dendritic contrast agent.

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Table 1. Biodegradable Dendrimer-Based MRI Contrast Agents

dendrimer	generation	chelate	ionic relaxivity[a] (mM^{-1} s^{-1})	author/ref
PE Dendron coupled to PCL–PEO polymersome	3	Gd(III)-DTPA	T_1 = 26.1*	Nazemi et al.(91)
PEG-conjugated EA dendrimer	-	Gd(III)–TACN–bis(1-Me)–3,2-HOPO–TAM–ethylamine	T_1 = 31; T_2 = 52**	Klemm et al.(92)
PE dendrimer	0–3	Gd(III)-DTPA	T_1 = 11.7***	Ye et al.(14)
FA PEG-conjugated PE dendrimer	2	Gd(III)-DTPA	T_1 = 17.1***	Ye et al.(89a)
PEG-conjugated PE dendrimer	1	Gd(III)-DTPA	T_1 = 10.2***	Li et al.(93)
PEG-conjugated PE dendrimer	2	Gd(III)-DTPA	T_1 = 10.7***	Li et al.(93)
PEG-conjugated PE dendrimer	4	Gd(III)-DTPA	T_1 = 15.6***	Li et al.(93)
PE dendrimer	5	Gd(III)-DTPA	T_1 = 17.5***	Li et al.(93)

[a] Measured at *20 MHz and 25 °C, **60 MHz and 37 °C, ***0.52 T and 32 °C.

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